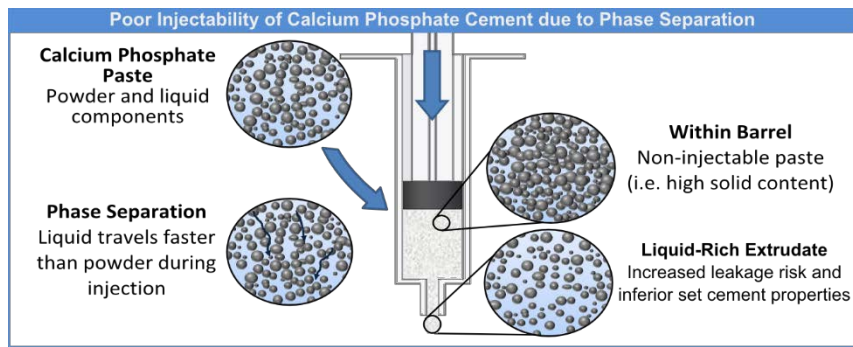


Graphical Abstract



Statement of Significance

Occurrence of phase separation of calcium phosphate pastes and cements during injection limits their full exploitation as a bone substitute in minimally invasive surgical applications. Due to lack of theoretical understanding of the phase separation mechanism(s), optimisation of an injectable CPC that satisfies clinical requirements has proven difficult. However, phase separation of pastes during delivery has been the focus across several research fields. Therefore in addition to a review of methods to reduce phase separation of calcium phosphate pastes and cements and the associated constraints, a review of phase separation mechanisms observed during delivery and the theoretical models used to describe these mechanisms is presented. It is anticipated this review will benefit future attempts to develop injectable calcium phosphate based systems.

Critical Review: Injectability of Calcium Phosphate Pastes and Cements

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Abstract

Calcium phosphate cements (CPC) have seen clinical success in many dental and orthopaedic applications in recent years. The properties of CPC essential for clinical success are reviewed in this article, which includes properties of the set cement (e.g. bioresorbability, biocompatibility, porosity and mechanical properties) and unset cement (e.g. setting time, cohesion, flow properties and ease of delivery to the surgical site). Emphasis is on the delivery of calcium phosphate (CaP) pastes and CPC, in particular the occurrence of separation of the liquid and solid components of the pastes and cements during injection; and established methods to reduce this phase separation. In addition a review of phase separation mechanisms observed during the extrusion of other biphasic paste systems and the theoretical models used to describe these mechanisms are discussed.

1 Introduction

Compounds of calcium phosphate (CaP) have been investigated as bone repair materials since 1920 [1]. However, they saw little use in clinical applications until the 1970s when CaP materials were used as bone substitutes in the form of porous blocks and granules [2–4]. The clinical potential of CaP materials further increased in the early 1980s with the development of self-setting calcium phosphate cement (CPC) [5]. In addition to its potential to mimic the mineral phase of bone, CPC has the ability to be moulded into bone defects and implant sites, then harden in situ to provide stability. This ability of CPC has shown great potential in percutaneous surgery whereby CPC is injected into the body to fill bone defects and stabilise fractures. Although CPC has shown clinical success in several orthopaedic applications requiring delivery by injection [6–12], it is thought that several issues currently prevent routine application in clinical applications. This has given rise to a high volume of studies aimed at improving the delivery of CPCs and broadening their clinical use [3].

Many of the studies attempting to optimise CPC for clinical applications focussed on improving the delivery of CPC to the surgical site through injection. A major issue inhibiting successful delivery of CPC is the occurrence of phase separation during injection. If phase separation occurs the extrudate has a higher liquid content than desired, which may cause extravasation from the surgical site and be detrimental to the final properties of the set CPC. The occurrence of phase separation during injection/extrusion of CaP pastes and cements, and methods to reduce it is the principal focus of this review.

Due to the high volume of studies published concerning CPCs, review articles have proven useful in presenting a summary of recent advances, highlighting current issues and opportunities within CPC research. The focus of recent comprehensive reviews

have included: processing techniques [13], mechanical performance [14], methods to reinforce CPCs [15,16], the role of polymeric additives [17], *in vivo* degradation and resorption of CaP materials [18], influence of CaP material properties on cell behaviour [19], CaP materials as drug delivery systems [20–22], stem cell delivery via CPC [23], and the synthesis and application of nanostructured CaP based materials [24–26], in addition to broader overviews of recent progress in the development of CPC materials [27–29].

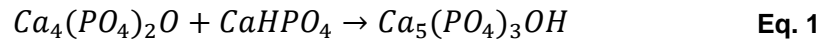
In this article established methods to reduce the phase separation of CaP pastes and cements and the limits of their application are reviewed. Brief discussions relating to the other crucial properties of CPC and their influencing parameters are also included as many established methods to reduce phase separation are detrimental to the other crucial properties, as evident throughout this review. Therefore, when optimising any property of CPC, it is important to consider all the crucial properties. In addition phase separation mechanisms observed during the injection or extrusion of other biphasic paste systems and the theoretical models used to describe these mechanisms are discussed. It is anticipated that including comparisons to work from fields outside of Biomaterials will give a new perspective and a greater understanding of the phase separation mechanism of CPC during injection, which will benefit researchers attempting to optimise a fully injectable CPC.

2 Types of Calcium Phosphate Cements

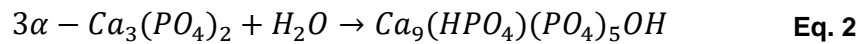
Due to the high level of interest and research into CPC, many different formulations of CPC have been developed. They can be divided into two principal groups: (1) apatite (hydroxyapatite, HA, and calcium-deficient HA, CDHA) and (2) brushite cements (dicalcium phosphate dihydrate, DCPD) [30]. Both apatite and brushite CPC are produced by mixing a powder component consisting of one or more calcium

orthophosphates with an aqueous solution. The mixing of these two phases induces the dissolution of the initial calcium orthophosphates. This is followed by precipitation into crystals of HA, CDHA or DCPD. During precipitation the newly formed crystals grow, and it is the entanglement of these new crystals, providing mechanical rigidity, that causes the cement to physically harden or set [29].

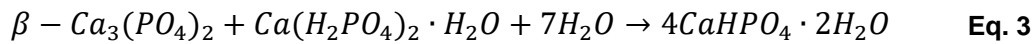
Hydroxyapatite (HA) can be formed via an acid-base reaction of tetra-calcium phosphate, TTCP (basic), and dicalcium phosphate anhydrous, DCPA (slightly acidic), Eq. 1.



Calcium deficient HA (CDHA) can be obtained via the hydrolysis of a metastable CaP e.g. α -tricalcium phosphate (α -TCP), Eq. 2.



Brushite (slightly acidic) can be obtained for instance by a reaction between β -TCP (almost neutral) and monocalcium phosphate monohydrate, MCPM (acidic), Eq. 3 [29].



Although α - and β -TCP have the same chemical composition, they differ in crystallographic structure, resulting in α -TCP being much more soluble than β -TCP [31]. Through thermal treatment above 650°C there are several methods used to produce β -TCP [31], which can transform to α -TCP at temperatures greater than 1125°C [32] (rapid quenching is required to prevent reverse transformation). A mixture of a α -TCP and β -TCP as the powder component and an aqueous solution of 2 wt% Na_2HPO_4 as the liquid part have been previously used to produce a CPC [33]. It was

found α -TCP fully hydrolysed to CDHA, whereas β -TCP remained unreacted and embedded in the CDHA matrix. Increasing the proportion of β -TCP phase resulted in an increased setting time and a reduction in the compressive strength of the set cement [33]. In fact pastes produced with only β -TCP and water are generally regarded to be non-setting and are used as a model paste system to represent unset CPC [34–36]. These pastes are referred to in this review article as ‘CaP pastes’.

3 Properties of Calcium Phosphate Cement

When designing CPC for orthopaedic applications the properties of the unset and set cement require careful consideration to ensure clinical success. The hardened cement must be biocompatible and have sufficient mechanical integrity to stabilise the fracture or implant site. Ideally the hardened CPC should have a suitable composition and adequate porosity to be bioresorbed and replaced by host tissue. The cement prior to setting has to be easily prepared and handled during the surgical procedure; for percutaneous surgical procedures such as treatment of fractures of the radius [9], tibia [10,12] or vertebrae through vertebroplasty (PVP) [8,11] and kyphoplasty (BKP) [37,38], this requires injection via a cannulated needle into the fracture site.

3.1 Bioresorbability and Biocompatibility

Animals studies have been used to examine the bioresorption (removal of material by cellular activity and/or dissolution of a material in a biological environment [39]) of CPC *in vivo*. In this article, several animal studies have been summarised (Table 1) to demonstrate the influence that type, size and porosity has on the bioresorbability of CPC. Further details of the bioresorption mechanism for CaP materials and the influence of physical and chemical properties of CaP materials on cell behaviour has

recently been presented in comprehensive reviews [18,19]. All cements considered in this article demonstrated good biocompatibility, as no signs of inflammation were detectable [40–43]. All cements were partially replaced by different amounts of new bone. However, variation in bioresorption rates was evident, Table 1. The variation of resorption may be attributed to inherent variation in animal studies, duration of the study, and also the type, size and porosity of CPC sample. Brushite CPC generally resorbs at a faster rate than apatitic based CPC, Table 1. This is due to the metastability of brushite in physiological conditions, i.e. brushite cement is not only resorbed by natural remodelling process but also by physiochemical dissolution [44]. The quantity of cement will also influence the resorption rate. In addition to size of sample, porosity will dictate the quantity of CPC. It can be observed that smaller (Knaak *et al.* [42]) and more porous (Miño-Fariña *et al.* [43]) samples have faster rates of resorption than larger less porous samples (Norian SRS tested by Apelt *et al.* [40] and Ooms *et al.* [41]), Table 1. Norian SRS demonstrated a lower porosity (~50% [45]) than the macroporous apatitic CPC investigated by Miño-Fariña *et al.* (75% [43]). However, the influence of porosity on the bioresorption mechanism of CPC is more complex than simply dictating the quantity of cement. Pore size and interconnectivity are also important influencing factors. As a result porosity of CPC has been the subject of several investigations, as illustrated in the next section.

3.2 Porosity

Porosity of the set CPC is closely related to the liquid-to-powder ratio, LPR, of the unset paste. As no, or little, water is consumed during the setting reaction, the majority of water or aqueous solution added to produce CPC is used as a dispersant medium to produce a workable paste [46]. A minimum amount of liquid is required to fill the voids between particles (this corresponds to the maximum solid volume fraction,

SVF_{\max}). The addition of liquid in excess of the minimum amount increases the particle-particle distance (reducing solid volume fraction, SVF) i.e. increasing LPR increases the porosity of the set cement.

It should be noted that water quantity consumed during the setting of brushite Eq. 3 is considerably higher than that of apatite Eq. 2. Therefore, at similar LPR, set brushite CPCs are generally less porous [47]. Using atomic weights, the mass of water consumed relative to the total mass of reactants in the setting reactions represented by Eq. 2 and Eq. 3 was estimated as 1.90 wt% (LPR 0.02) for apatite and 21.25 wt% (LPR 0.26) for brushite.

Although the microstructure of CPC based systems are porous, their porosity lies within the high nano to sub-micron range. This enables fluid flow within the microstructure of the cement and increases the surface area, leading to greater surface reactivity [48]; however, the pores are typically considered too small to facilitate tissue ingrowth [49]. As a result, resorption of CPCs generally occurs layer-by-layer (i.e. from the outside to the inside) [31]. Interconnecting macroporosity enhances bone regeneration mechanisms [50] and improves apposition to host bone, allowing stabilisation of the defect and improves the healing process. Methods used to enhance macroporosity of CPC include the addition of porogenic agents, such as mannitol [51] or sucrose [52], the use of gas generating compounds, such as sodium bicarbonate [52,53], or the use of foaming agents such as albumen [43,50], gelatine [54], soybean gelatine combinations [55] or low molecular weight surfactants [56]. Even with these methods, the process for reliably controlling the pore structure of CPC during percutaneous surgery is difficult, especially when compared to 3D printing methods used to produce CaP scaffolds prior to implantation. Nevertheless, knowledge of the pore structure is essential and, as a result, investigations into

accurate measurement of the pore size and morphology of CPC have been conducted [57–59]. However, with regards to increasing porosity of CPCs for hard tissue replacement, there is a major limitation — mechanical properties are strongly dependent on porosity and they decrease exponentially with an increase in porosity [31].

3.3 Mechanical Properties

Compressive strength is the most common, and often the only criterion [14], used to assess the mechanical performance of CPCs. Compressive strength of CPC can be increased by reducing porosity or via the introduction of additives, such as citric acid [60] and superplasticisers [61]. In addition, apatitic CPCs generally have greater strength than brushite [14]. When optimising the strength of CPC, or selecting a commercial CPC for orthopaedic applications, it would make sense for the compressive strength of the cement to be similar to the compressive strength of bone. However the compressive strength of bone can vary widely, depending on age, gender, location within the body and the stress imposed on it. Bone adapts to the stresses imposed on it by remodelling, varying in size and altering the abundance of cortical (providing strength) and cancellous (reducing weight) bone.

Therefore, depending on the clinical application, the required compressive strength will vary; i.e. the required compressive strength for filling and stabilising fractures at load bearing anatomical locations such as tibial plateau [10] or vertebrae [8,11] would differ to CPC used to fill defects in maxillofacial surgery applications [62].

To investigate the effect PVP has on the strength of vertebrae, Lim *et al.* [63] and Hong *et al.* [64] evaluated the strengths of vertebral bodies pre-fracture and post-PVP. Vertebrae retrieved from human cadavers were subjected to compressive loading until

failure. Post failure, the vertebrae were injected with cement to simulate PVP. The cements used included CPC and PMMA. Lim *et al.* [63] found that the maximum compressive load (MCL) of the vertebrae was higher post PVP compared to MCL values pre-surgery. This was true when CPC or PMMA was used, although not always significantly with regards to CPC [63]. Hong *et al.* [64] also found vertebrae treated with PMMA bone cement demonstrated an increase in MCL. However vertebrae treated with CPC showed a decrease in MCL; this decrease was not statistically significant for all cases (Table 2). In both studies, MCL values of vertebrae treated with CPC were closer to the vertebrae prior to operation when compared to PMMA.

Although studies have demonstrated that CPC can be used with PVP to improve the strength of a fractured vertebra to its pre-fractured strength [63], it has been stated that compressive strength alone is not a sufficient criterion to assess mechanical properties of CPC [14]. Other essential mechanical properties have been investigated, including fracture toughness [45,65,66] and fatigue behaviour through cyclic loading [45,65,67], but these have received less attention and should be the focus of future mechanical performance investigations [14]. The environment in which cements are tested should also be considered [68]. Generally mechanical testing of CPC is normally conducted under dry conditions at room temperature. However, testing under wet conditions at body temperature would be more representative of the *in vivo* scenario. Indeed the mechanical strength of several CPCs tested in both wet and dry conditions have been compared [68]. It was found that the mechanical strength was lower when tested under wet conditions with the exception of one CPC which exhibited no significant change in compressive strength [68].

However the major issue in future investigations attempting to optimise the mechanical properties of CPC will remain, i.e. improving mechanical properties without sacrificing

other essential properties. For example, an investigation into fracture toughness of CPC showed the addition of collagen fibres improved the fracture toughness, but this addition also reduced the compressive strength and inhibited delivery of the cement through a cannulated needle [66].

4 Properties and Delivery of Unset Calcium Phosphate Cement

The method by which the CPC is delivered to the surgical site, can directly impact the properties previously discussed in Section 3.1 to Section 3.3. The unset CPC should be easily loaded into a syringe and injected into the body to the surgical site through a cannulated needle. The needles used in percutaneous surgeries such as PVP and BKP usually range from 13 to 8-gauge (internal diameters of 1.80 to 3.43 mm) [69] and from 100 to 150 mm in length [70]. The needle geometry required may be considerably smaller than this, depending on the surgical application. For example, during the manufacture of tissue engineered scaffolds via direct deposition methods, CPC has been extruded through 27 to 23 gauge needles (internal diameters of 0.16 to 0.32 mm) [71]. The rheological properties of the paste should be sufficient to allow injection through the cannulated needle at a force that can be applied by an orthopaedic surgeon (usually taken as 100 N [70] to 300 N [60]) and fill the fracture site, yet not cause extravasation. In addition, the CPC should have sufficient cohesion to prevent disintegration in the body. There is evidence that disintegration, due to poor cohesion, of CPC in the body during PVP can lead to cement embolism [71,72], with potentially fatal consequences [71].

A major issue in cement delivery is the occurrence of separation of the powder and liquid components of CPC during delivery using a syringe. This occurs when the liquid phase travels at a faster rate than the powder particles, resulting in extrudate with a

significantly higher liquid content than the paste loaded into the syringe [34]. A higher liquid content leads to a decrease in the viscosity of the unset CPC [73], an increase in setting time [74], a reduction in cohesion [75] and a decrease in the mechanical strength of the set CPC (Section 3.3). Non-extrudable paste remaining in the syringe, due to a low liquid content, is also a consequence of phase separation [34].

The extent of phase separation occurring during extrusion of CPC is commonly inferred from a measurement called '*injectability*' [34]. Injectability is usually defined as the mass of extrudate relative to the mass of the initial paste. Poor injectability of CPCs is a major factor limiting their application in PVP and BKP surgical procedures [76,77]. As a result, many studies have focused on modifying the constituent parts of CPC to improve injectability; indeed, many improvement methods have been established.

5 Improving Injectability of Calcium Phosphate Cement

Phase separation of CPC is believed to be a result of high extrusion pressure relative to liquid filtration pressure [78]. Improvement methods would therefore include: (i) reducing the extrusion pressure, i.e. increasing the flowability (reducing the pressure required to cause the paste to flow), and (ii) increasing the pressure (P_{PS}) required to force the liquid through the powder network, i.e. reducing the permeability (k) of the paste system.

$$P_{PS} = \frac{Q\mu_l L}{kA} \quad \text{Eq. 4}$$

Where Q and μ_l are flow rate and viscosity of the liquid component, and L and A are the length and area of the powder bed.

Many methods to improve injectability have been established, which will be discussed with reference to their effect on flowability and permeability of the paste system.

5.1 Viscosity of Liquid Phase

One method that has been shown to improve injectability of CaP based cement and paste is increasing the viscosity, μ_l , of the binder. This increases P_{PS} (Eq. 4) and has been demonstrated to reduce [35,79] and even eliminate [76,80–82] phase separation. Viscous binders that have been investigated include aqueous solutions containing sodium hyaluronate [35], methylcellulose (MC) [79], hydroxypropyl methylcellulose (HPMC) [76,79,81], salts of alginic acid [82] and ethylene oxide/propylene oxide block copolymers such as Pluronic [80]. Contrastingly, Wang *et al.* [83] reported that the viscous gel produced with the addition of modified starch to CPC reduced injectability. However, in their study the modified starch was added to the powder to form the solid phase of the CPC before the addition of water [83].

Although viscous binders have had favourable results with regards to reducing phase separation, they did present certain limitations. For example, increasing the concentration of Pluronic increased the extrusion force [80], possibly due to reducing the flowability of the bulk paste. A paste that will not readily flow may be desirable in applications such as robocasting of scaffolds, the application of interest to Franco *et al.* [80], as it increases the likelihood of the scaffold maintaining its shape during setting. However, it may not be suitable for percutaneous surgery as it will inhibit filling of the fracture site with cement. It was also observed that the cements produced with MC, HPMC and modified starch had significantly lower compressive strengths than cement that used water as the liquid phase [79,83]. This has been attributed to an

increase in porosity due to air bubbles trapped in the CPC during production of the paste when viscous binders are used [79].

5.2 Maximum Packing Fraction of Powder and LPR

Optimising the solid phase of the paste may also improve injectability by altering the powder permeability and maximum packing solid volume fraction (SVF_{max}). The SVF_{max} of the powder component of pastes plays a significant role in the flowability of that paste. To produce a paste from powder and liquid components, the liquid has to first fill the voids of the powder matrix or network: any liquid in excess of this amount will then increase particle-particle distance and initiate flow (the greater the excess, the more readily the paste will flow). Therefore, the greater the deviation between SVF of the paste and SVF_{max} of the powder component the greater the flowability. Increasing SVF_{max} of powder and reducing SVF (i.e. increasing LPR) of the paste are methods to increase flowability. Indeed, increasing LPR has been seen to improve injectability of CPC significantly [70,76]. However, increasing the LPR will also increase permeability of the paste system and reduce the mechanical strength of the set cement due to an increase in porosity (Section 3.3), and is therefore limited as a CPC improvement method. For this reason increasing SVF_{max} is an appealing method to increase the flowability of CaP pastes. Powder SVF_{max} and permeability can be optimised by altering the powder morphology including: (i) particle size, (ii) distribution and (iii) shape.

(i) Particle Size

Theoretically, particle size should not affect packing ability of powders. Hales *et al.* [84] proved Kepler's conjecture that the SVF_{max} for hard spheres is, $\frac{\pi}{\sqrt{18}} = 0.74$, independent of size; although it is widely accepted that the random SVF_{max} of mono-

sized spheres is approximately 0.64 [85]. However, in practice, reducing particle size may reduce SVF_{max} , due to agglomeration, as observed with CPC comprising of relatively finer particles [86]. Indeed, reducing particle size has been shown to increase viscosity of CPC [73]. However, a reduction in particle size would decrease permeability due to an increase in surface area, beneficial to enhancing injectability. In general, a smaller particle size has been demonstrated to improve injectability of CaP pastes [78,87] and CPCs [30,87]. In one study, although the general trend was that a reduction in particle size increased injectability, it was also observed that adopting excessive cryogenic and ball milling times had a detrimental effect on injectability [30]. This was attributed to agglomeration of particles [30]. This indicates there may be a limitation when using milling protocols to reduce the particle size of CaP powder and increase injectability.

(ii) Particle Size Distribution

Broadening the size distribution of powder by adding particles smaller than those in the bulk powder, filling the voids (Fig. 1B, C), is a common way to increase SVF_{max} . This also reduces permeability of the powder. The proportion of fine to coarse powders can be optimised to produce the highest achievable SVF_{max} , (Fig. 1C). Too low a quantity of fine particles will not sufficiently fill the voids, (Fig. 1B); too high a quantity will not allow the large particles to form a network (Fig. 1D). Again, in theory the SVF_{max} of the small particles (Fig. 1E) should be equivalent to that of the coarse powder (Fig. 1A) [85]. Several models exist to estimate the SVF_{max} of powder mixtures, including the Furnas' model [88] to estimate the SVF_{max} of binary mixes Eq. 5i and 5ii.

$$SVF_{maxb} = \frac{SVF_{maxc}}{1 - VF_f} \quad \text{Eq. 5i}$$

$$SVF_{maxb} = \frac{SVF_{maxf}}{VF_f + SVF_{maxf} - VF_f SVF_{maxf}} \quad \text{Eq. 5ii}$$

Where SVF_{maxm} , SVF_{maxf} and SVF_{maxc} are the SVF_{max} of the binary mixture, and the fine and coarse components, VF_f is the volume fraction of fines in the mixture. The locus of the Eq. 5i and 5ii intersect, Fig. 2. The intersect represents the optimum VF_f to achieve the highest SVF_{maxm} , values above the intersect should be ignored [89].

The above discussion applies to cases where the difference in diameter of the original and added particles is sufficiently large that the added particles do not disrupt the packing structure of the original particles. If this is not the case the addition of different sized particles may increase the voidage. For example, if fine particles (outlined in red in Fig. 3) are not small enough to fit into interstices of coarse particles, the packing structure is disrupted, increasing the voidage. This is described as the *loosening effect* [90]. The voidage can also be increased if a particle with a larger (yet comparable) diameter is added (outlined in black in Fig. 3). As this has a similar effect on packing as a container wall may have, this is referred to as the *wall effect* [90].

With regards to CaP powders; Gbureck *et al.* [91], altered the packing ability by the addition of fine fillers, which reduced the water demand and improved injectability (Table 3). Qi and Ye [92] and Tadier *et al.* [35] added larger beads to CaP powders. Qi and Ye [92] found the addition of larger beads decreased injectability (Table 3). Tadier *et al.* [35] found the addition of beads with a diameter of 156 μm improved injectability. However the addition of 390 μm beads, had little detrimental effect on injectability depending on the wt% of beads (Table 3).

In the studies by Gbureck *et al.* [91], Tadier *et al.* [35] and Qi and Ye [92], as the added particles were different in material than the bulk powder, it is difficult to determine if the observed effects were solely due to altering the SVF_{\max} . Alternatively, some mechanism affecting the particle-particle interaction may have disrupted the solid network within the pastes. In addition, Torres *et al.* [77] investigated the injectability of pastes produced from β -TCP powders of various size and distribution, and demonstrated that particle size distribution (PSD) did influence injectability. The effect of PSD on packing ability was discussed, however methods to estimate the specific influence of median size, breadth of distribution or shape on packing ability were not conducted [77].

(iii) Particle Shape

Particle shape has been shown to affect injectability, with spherical CaP particles having better injectability than irregular particles [93]. Although permeability of powder will increase with an increase in sphericity, it is generally observed that particles with higher sphericity will have higher packing abilities [85]. This could be the reason for the observed increase in injectability in many studies. It should be stated that particles with other regular geometric shapes (e.g. cubes and parallelepipeds) have been observed to achieve higher SVF_{\max} than spheres [85]. However, these shapes would be difficult to achieve in normal CaP powder production processes.

5.3 Particle-Particle Interactions

Particle-particle interactions dictate the strength of the powder network and will therefore influence the flowability of pastes. Two particle interactions within concentrated CaP pastes and cement will be considered: (i) colloidal interactions and (ii) direct frictional contact.

(i) Colloidal Interactions

It has been stated that in liquid-powder systems colloidal interactions become significant when particle diameters are less than 40 μm and very significant at diameters below 10 μm [94]. As CPC particles are generally below 10 μm , colloidal interactions should be considered when investigating the flowability of unset CPC. Although there may be several complex forces influencing the interaction of colloidal particles in aqueous environments [95], generally forces are simplified to the sum of the attractive Van der Waals and the repulsive electrical double layer (EDL), defined in the Derjaguin, Verway, Landau and Overbeek (DVLO) theory [96,97], Fig. 4A. The EDL is formed due to the charge at the particle surface-liquid interface. To maintain neutrality counter ions are attracted to the particle surface [98]. The counter ions form two layers, (i) the stern layer and (ii) diffuse layer Fig. 4B. If the EDL of two particles overlap a repulsive force between the particles develops.

At an increasing distance away from the particle surface, the potential decreases linearly through the stern layer and exponentially through the diffuse layer reaching zero at the bulk solution [99], Fig. 4B. A common laboratory measurement to estimate the magnitude of the surface potential is the zeta potential, ζP . The ζP is the potential at the slipping or shear plane, where counter ions within this boundary move with the particle, Fig. 4B. The greater the ζP the more stable the suspension, and with regards CPC pastes it has been observed the greater the ζP the greater the injectability [60].

Gbureck *et al.* found ζP of TTCP and DCPA in water was -18.4 ± 1.9 mV and -15.0 ± 1.8 mV, whereas the ζP for the same powders in a trisodium citrate solution was -50.1 ± 1.0 mV and -50.6 ± 3.8 mV [60]. Consequently a CPC produced with TTCP, DCPA and water achieved an injectability of 59%, while under the same conditions, a

CPC produced with TTCP, DCPA and trisodium citrate solution achieved an injectability of 97.4%. However, increasing the magnitude of ζP is limited as an improvement method due to the detrimental effects that reducing particle attraction may have on cohesion [75].

(ii) Direct Frictional Contact

Direct frictional contact between particles within concentrated pastes are strongly dependent on roughness and SVF [100]. Methods reducing frictional contact between particles, including increasing LPR [70,76], have been shown to improve injectability. Ishikawa *et al.* [93] observed spherical particles exhibited better injectability than irregular particles, which was attributed in part to smoother surfaces. Leroux *et al.* [101] observed improved injectability when glycerol was added, due to the lubricating effect of the binder reducing frictional contact. Alternatively, this may have been due to a reduction in permeability, as the glycerol would be more viscous than water.

5.4 Setting Reaction

Particle interaction is also made more complex with the addition of a setting reaction. As needle like structures form at the surface, surface roughness increases and a porous solid forms due to the mechanical interlocking of the crystals. Thus, the setting reaction has often been observed to have a detrimental effect on the injectability of CPC [70,87,101]. The most common method of monitoring the setting reaction of CPC is using the Gillmore needle apparatus. This technique determines the time at which the setting cement can support a static pressure of 0.3MPa (initial setting time, t_i) and 5MPa (final setting time, t_f) without being deformed. With regards to clinical applications the surgeon should be able to prepare and deliver the CPC to the surgical site before t_i is reached [102]. Depending on the surgical procedure it has been

suggested that t_i should be between 3 and 8 min, close to 3 min for dental related procedures and close to 8 min for orthopaedic procedures, t_F should be less than 15 min [102]. Khairoun *et al.* [70] proposed a new term, relating the setting reaction to injectability, referred to as dough time, t_D , defined as the time at which injectability reaches 0%. Some discrepancy has been observed in t_D , however. Khairoun *et al.* [70] observed that t_D was approximately half of t_i , while Montufar *et al.* [87] observed 0% injectability (t_D) was not reached until well after the initial setting time, Fig. 5. One reason for the different relationships between t_i and t_D in these two studies could be factors other than the setting reaction that influence injectability were not kept constant. The paste systems most likely differed in permeability and flow properties — therefore, the extent of phase separation would have varied between the studies. As discussed previously, phase separation causes the paste remaining in the barrel to dewater. It has also been established that reducing the LPR of CPC reduces the setting time [87]. Therefore, the setting time measured for the composition of CPC initially loaded into the syringe is not the setting time exhibited by the paste within the syringe during extrusion if phase separation occurs. This highlights that, when attempting to optimise setting times of CPC for surgical applications requiring injection or extrusion, it is crucial to consider if, and to what extent, phase separation occurs. Currently the most notable method used, attempting to reduce the influence of the setting reaction on the delivery process, is the development of premixed CPC that does not set until injected into the body [103–106].

5.5 Extrusion Parameters

Extrusion parameters have also been shown to significantly affect injectability. Habib *et al.* [34] observed injectability of a CaP paste was reduced when larger syringes were used and with the addition of a needle. However, Montufar *et al.* [87] reported no

significant difference between the extrusion of a CaP paste with or without a needle, but did observe needle geometry affected injectability of CPC. Increasing extrusion rate, reducing the time for liquid to migrate [107] has been shown to increase injectability [34]. O'Neill *et al.* observed that modifying the syringe geometry by adding a taper at the barrel exit also significantly increased injectability [36]. Additionally, agitating CaP paste throughout extrusion by applying ultrasonic vibration also significantly improves injectability [108]. However, further investigation is required into the effect the amplitude of vibration has on the tip of the needle, which may have detrimental effects during PVP [108]. The manner by which the unset CPC is loaded into the syringe and mixed beforehand will also influence phase separation as mixing and loading will dictate the uniformity of liquid distribution throughout the unset CPC prior to injection. Therefore, it is important that the mixing method used is sufficient for a uniform liquid distribution to be achieved, which should not be altered during loading. The energy of mixing (duration and power) may also alter the flow properties of CPC and affect the injectability – even though the duration of CPC mixing is limited due to the setting reaction. However, to the best of the authors' knowledge there have been no studies investigating the influence of mixing on the delivery of CPC based systems reported. Therefore, investigations into the influence of different mixing approaches (using low, medium- and high-energy regimes) in order to understand its influence on flow properties of CPC and consequently its injectability should be conducted in the future. Until then, it should be highlighted that research relating to the mixing of construction cements is well established and it has been reported that such cement when subjected to high shear mixing, demonstrate improved flow properties compared to the same cements, when subjected to low shear or hand mixing [111]. Due to the

similarities between CPC and construction based cements it is likely a similar effect would be exhibited for CPC when subjected to relatively higher shear mixing regimes.

5.6 Summary of Improvement Methods

It is apparent that several methods have been established to improve injectability, and even produce fully injectable CPC in some cases, and injectable CPC based systems have had clinical success in several applications [6–12]. Yet, it is widely accepted that there are still many issues inhibiting the optimisation of an injectable CPC that fully satisfies clinical requirements [14,109]. A major issue is the fact that many of the methods to improve injectability are detrimental to other crucial properties of CPC and vice versa. Therefore, to produce a CPC within the constraints of a considered clinical application, a combination of improvement methods is required. Due to the number of independent and dependent variables to consider, optimisation of a CPC based system through experimental work alone has proven difficult and time consuming. This is evident from the large number of studies, conducted for over two decades, attempting to improve CPC. Thus, greater theoretical understanding of how, and to what extent, process variables affect the crucial properties of CPC is required.

6 Theoretical Consideration of Injectability for Calcium Phosphate Paste

Bohner and Baroud [78] applied a theoretical approach to investigate the effect of several variables on injectability of a CaP paste. They developed a model that combined the Hagen-Poiseuille equation (Eq. 6), describing the flow of the CaP paste through a cannulated needle, and a derivative of Darcy's law (Eq. 7), representing the filtration of the liquid phase through the powder network:

$$P_i = \frac{128\mu_p L_n Q_p}{\pi D_n^4} \quad \text{Eq. 6}$$

where P_i is injection pressure, L_n and D_n are length and diameter of the needle, and Q_p and μ_p are the flow rate and viscosity of the paste, respectively.

$$th^2 = \left(\frac{D_e^2}{150\mu_l} \frac{\varepsilon_m^3}{(1 - \varepsilon_m)^2(1 - \varepsilon)} P_c \right) t \quad \text{Eq. 7}$$

Eq. 7 is concerned with cake filtration, i.e. the filtration of a liquid through a dense powder bed. Using Eq. 7 the thickness of the bed, th , is estimated based on the diameter of particles, D_e , the voidage (water volume fraction) of the paste and cake, ε and ε_m , time, t , the pressure drop through the cake, P_c , and the viscosity of the liquid, μ_l .

It was assumed that P_c is equal to P_i , and μ_p can be described by the Quemada result (similar in form to the Krieger Dougherty equation, Eq.8).

$$\mu_p = \mu_l \left(1 - \frac{SVF}{SVF_{max}} \right)^{-2} \quad \text{Eq. 8}$$

It was assumed that $1 - \varepsilon_m = SVF_{max}$ and SVF_{max} was related to plastic limit (PL), which is the minimum LPR at which a 'pasty block' is formed. Upon converting the volume fractions (SVF , ε and ε_m) to mass ratios (LPR and PL) Eq. 7 becomes,

$$th^2 = \left(d_e^2 \frac{128}{150\pi} f(PL, LPR) \frac{L_n}{D_n^4} Q_p \right) t \quad \text{Eq. 9}$$

where,

$$f(PL, LPR) = \frac{\rho_s PL^3 (1 + \rho_s LPR)^3}{(1 + \rho_s PL)(LPR - PL)^2} \quad \text{Eq. 10}$$

Eq. 9 could not be directly corroborated with experimental results. Instead it was validated by establishing if factors decreasing cake growth were the same factors increasing injectability in experimental investigations. The positive effects of increased LPR and decreased particle size observed experimentally were predicted by the model. However, experimentally it was found that increasing the viscosity of the liquid improved injectability, which was not predicted using the model [78]. This was probably due to the relative simplicity of the method used to deal with the pressure components. In the actual extrusion process of CaP paste, the required extrusion pressure would be the sum of various pressure components, i.e. pressure forcing the paste through the barrel exit (divided into pressure acting on the liquid and powder network) and pressure to overcome paste-wall friction. However, in this model it is simply assumed that P_c equals P_i . In replacing P_c Eq. 7 with the right hand side of Eq. 6 and using the Krieger Dougherty equation to predict μ_p , μ_l was removed and not represented in the model. The model also predicted a larger th , with a decrease in d_n ; however, injectability did not change considerably when altering the needle diameter during experimental investigations. This was thought to be due to a limited number of data [78].

7 Limitations of ‘Injectability’ as a Measurement

The model proposed by Bohner and Baroud [78] was the first attempt at relating important characteristics: namely, rheological and permeability properties and extrusion parameters of the paste system to injectability and phase separation of CaP materials. In doing so, Bohner and Baroud highlighted the lack of knowledge across these areas. Although dozens of investigations have been performed, the exact mechanism of phase separation and its effect on the permeability and flow properties of the CaP pastes throughout the extrusion process remains unknown. The reason

for this, in part, lies with measurement of 'injectability'. Injectability is only a measure of one resulting aspect of a complex process revealing little of the process or the characteristics of the paste systems used. With this limited measurement and no standard testing protocol (e.g. syringe geometry, extrusion rate and force, particle size and LPR all differ between studies), it is very difficult to determine the benefits of one improvement strategy versus another by cross-comparing separate studies. Furthermore, injectability does not take into account the quality of extrudate. Without analysis of the extrudate the effect injectability improvement methods have on the quality of extrudate remains unclear. Therefore, increasing injectability may not necessarily mean increasing clinical applicability. It is for this reason that Bohner and Baroud [78] proposed injectability should be defined as the capacity of the CPC system to stay homogeneous during the injection process.

Consequently, Habib *et al.* investigated paste homogeneity during extrusion by measuring and comparing the LPR [34] and PSD [110] of extrudate and paste remaining in the syringe post-extrusion. No particle size segregation was observed during extrusion [110]. However, phase separation was confirmed as the extrudate LPR was higher than that of the initial paste. In addition, the paste remaining in the barrel showed a considerable decrease in LPR, resulting in an increase in required extrusion pressure [34]. Higher extrudate LPR compared to initial LPR was also reported by Tadier *et al.* [35] and O'Neill *et al.* [36] when investigating the extrusion of CaP pastes. Although phase separation was confirmed as the factor limiting injectability, the exact mechanism remains unknown.

8 Permeability of Calcium Phosphate Powders

Habib also investigated the correlation between injectability and permeability properties of CaP pastes [111]. Using the Blaine apparatus (ASTM C:204), Habib determined the permeability of β -TCP powders of various PSD [111]. To vary the PSD, finer plasma treated β -TCP and HA powders at various wt% were added to a commercial β -TCP powder. It was observed that decreasing voidage and increasing the wt% of fines (HA powder, 3 wt% max. and plasma treated β -TCP, 10 wt% max.) reduced permeability. To establish if a correlation between permeability and injectability existed, Habib compared the permeability values to injectability values obtained from pastes produced using the same powder mixtures at an LPR of 0.4. It was found that permeability values obtained were not a strong predictor of the liquid separation phenomenon [111].

The study by Habib was the only study found investigating the permeability of CaP powders and relating it to the phase separation of CaP paste during extrusion. In addition, the SVF of powder used to test permeability (0.355 to 0.325) in the study was lower than SVF of pastes that were used to investigate injectability ≈ 0.45 . Further investigation into the effect permeability has on phase separation is thus required [111].

9 Rheology of Calcium Phosphate Pastes and Cements

The rheological properties of dilute suspensions are relatively well understood. In contrast, concentrated suspensions and pastes with a relatively high SVF, such as CPC for orthopaedic applications, exhibit an apparent viscosity far higher than that of the liquid component due to complex particles interactions. The rheology of these materials have received less attention and are therefore less understood [112].

Due to the complexity and inherent heterogeneity of pastes it is difficult to obtain reliable and physically meaningful measurements from the rheometry of pastes [113]. The heterogeneity causes problems including liquid migration, sedimentation, wall slip, cohesion failure, and localised shear, which can lead to serious errors in the measurements [112]. For these reasons it is very tempting to use established semi-empirical expressions to predict viscosity of suspensions, like the Krieger-Dougherty equation [114], or those proposed by Dabak and Yucel [115] and Liu [116]. Models to predict the yield stress of concentrated pastes have also been proposed [117]. However, these models only consider a portion of the factors influencing the rheological properties, relying on empirically derived parameters and ‘must be seen as only rough approximations of reality’ [100]. Therefore, overcoming the difficulties in performing rheometry of CPCs and pastes is essential to better understand the flow properties of the cement and the influencing factors.

A common method to determine the flow properties of CaP pastes and cements is measuring the plastic limit, i.e. the quantity of liquid added to a powder to produce a pasty block [78,91,118]. It is a measure of water demand that can be used to infer the SVF_{max} of powder or flowability of pastes, as used by Bohner and Baroud in their theoretical model [78], the assumption being lower plastic limit leads to more flowable the paste. Indeed, plastic limit, specifically a function of plastic limit and initial LPR, has been observed to be a strong predictor of injectability [34,78]. However, the measure of plastic limit is quite subjective and does not determine how the paste will perform under flow conditions.

Rheometers have been used to investigate how various CaP suspensions, pastes and cements perform under flow conditions [73,119–125]. Baroud *et al.* measured the rheological properties of a CaP paste (β -TCP and H_2O) [73] using a rotational parallel

plate rheometer at various increasing shear rates (from 0 to 200s^{-1}). It was observed that increasing LPR and reducing milling time (also resulting in larger particles sizes) reduced viscosity. This was also observed by Friberg *et al.* during the rheometry of CPC (α -TCP and H_2O) [125]. The addition of a flocculating modifier (e.g. xanthan) and a deflocculating modifier (e.g. sodium polyacrylate) was investigated: xanthan increased viscosity and sodium polyacrylate decreased viscosity [73]. All formulations tested displayed a yield stress and shear thinning characteristics [73].

Liu *et al.* used a rotational cone and plate rheometer to measure the flow properties of a CPC [123]. Their study focussed on the evolution of the particle network during the setting reaction; however, steady rheology was also investigated. A yield stress, shear thinning and thixotropic characteristics were evident, Fig. 6.

Yield stress, shear thinning and thixotropic characteristics are commonly exhibited by concentrated pastes. The presence of these characteristics demonstrated the significance of the particle network with regards to the flowability of CaP pastes and cements. A yield stress suggests the sum of the interaction forces between particles is attractive, and this force and SVF are sufficiently high that the particles can create a continuous network. A certain shear stress is required to overcome the interparticle attractive force, breaking the network and inducing flow. Shear thinning of concentrated pastes and cements is attributed to the particle network being further broken or destructured as shear rate is increased, reducing the apparent viscosity. Thixotropy also involves the structural breakdown of the powder network. However, it is not only dependent on the instantaneous shear rate, but also the shear history. In addition thixotropy is reversible as the attractive bonds can reform when the paste is at rest. In Fig. 6, the apparent viscosity on the return sweep is lower than the outward

sweep, due to breakdown of the particle network. Over time, at rest, the structure will reform and viscosity increase.

In addition to the rheometry studies by Baroud *et al.* [73] and Liu *et al.* [123], several studies have performed both injectability tests and rheometry for various CaP paste and cement formulations [34,77,83,87,92,126]. Habib *et al.* [34] varied LPR of pastes produced with β -TCP. Torres *et al.* also investigated the effect LPR had on CaP pastes, in addition to various calcination temperatures and milling times [77]. Several studies have investigated the effect various additives had on the rheology of CaP pastes and cements: precipitated HA nanoparticles [87], chitosan, sodium alginate, modified starch [83], PLGA microspheres [92], polyethylene, glycerine, citric acid [126], and Na_2HPO_4 [87,126]. In general, it was found that methods to reduce viscosity increased injectability. However, this trend was not always observed. Torres *et al.* [77] using various pastes produced with β -TCP powders of different PSD observed that injectability was more sensitive to variations in PSD than the viscosity of the bulk paste alone (measured using a cone and plate rotational rheometer) [77].

Permeability is an obvious factor that influences phase separation and is dependent on PSD, but other unknown mechanisms may also be occurring. Therefore, when investigating phase separation, the permeability and rheological properties of the paste system should both be considered. This will enable the extent these properties affect phase separation to be investigated and also assist in the identification of any other mechanisms contributing to phase separation during extrusion. Indeed, although several studies have conducted both rheometry and injectability tests on CaP pastes and cements [34,77,83,87,92,126], there appears to be no attempt to theoretically or empirically determine the extent by which measured yield stress or viscosity affects injectability. Furthermore, comparison of flow properties of CaP cements and pastes

between the studies discussed is also limited, as generally only viscosity or shear stress versus shear rate profiles have been reported.

9.1 Comparing Rheological Properties of Pastes

Commonly, flow properties of various pastes and fluids are compared between studies by using constitutive models, via flow curve fittings, to obtain key parameters. These parameters can also be utilised in theoretical models. Fatimi *et al.* used the Ostwald–de Waele equation (Power law) Eq. 11 to obtain key parameters from the rheometry of a CPC (i.e. biphasic CaP and HPMC aqueous solution) [81]. The parameters were used with the Hagan-Poiseuille equation to estimate the extrusion pressure for the CPC. As the CPC was fully injectable, phase separation was not considered:

$$\tau = k\dot{\gamma}^n \quad \text{Eq. 11}$$

where k is the consistency parameter and n the shear rate index.

The Ostwald–de Waele equation does not take into account the yield stress; however, the existence of a yield stress has been observed during several CaP rheometrical investigations [34,73,83,92,126]. A common and established model, similar to the Ostwald–de Waele model, which includes yield stress is the Herschel-Bulkley equation:

$$\tau = \tau_0 + k\dot{\gamma}^n \quad \text{Eq. 12}$$

where τ_0 is the yield stress.

The Ostwald–de Waele and Herschel-Bulkley equations are very useful in identifying flow characteristics. If $n < 1$ the material is shear thinning. If a linear relationship exists between τ and $\dot{\gamma}$ once yield stress is reached (i.e. $n = 1$), the material is referred to as

a Bingham plastic. Finally, if $n > 1$, the material is shear thickening, Fig. 7. Note Fig. 7 does not include an time dependency.

In the construction industry, constitutive models have been of great benefit when comparing flow properties of various cement formulations. However, it has been observed that parameters obtained can vary vastly [127,128]. It is intuitive, due to complex flow characteristics such as shear thinning and thixotropy that parameters representing the viscous component of cement may vary between studies, depending on the rheometry device or protocol used. These complexities can also affect the measurement of the yield stress; methods used to prepare, mix and deliver the cement, how it is loaded into the device and any pre-shearing protocols the cement is subjected to before measurement will all affect the measured yield stress value. A review by Banfill [128] demonstrates very well the difficulties encountered when attempting to compare yield stress values for cement based pastes from different studies. Banfill found that reported yield stress values vary widely for construction cements, which are not sufficiently different in chemical composition or PSD to warrant the variation in measured yield stress observed [128].

Even studies comparing the testing of identical cement pastes on different rotational rheometers found results differed considerably [129,130] (even over an order of magnitude [127]). Therefore with regards to CaP pastes and cement, care has to be taken when comparing inter-laboratory rheometry results. Although measured material properties should be ideally independent of the measuring device, this is rarely the case for rheometry of cement-like paste. To address this, Ferraris *et al.* [131] recently proposed a reference or calibration paste to use in rheological studies of cement-like pastes, which may prove useful in future rheometry investigations. When conducting rheometry investigations for homogeneous fluids standard reference oils with known

rheological parameters can be used to test the reliability of the rheometer, geometry and protocol used. However, due to the previously discussed complexities associated with rheometry of biphasic pastes, there is more opportunity for erroneous measurements when compared to rheometry of homogeneous fluids. Therefore, achieving accurate rheometry measurements for standard reference oils does not necessarily mean that accurate and reliable measurements can be obtained for biphasic pastes using the same protocol. Ferraris *et al.* [131] have developed a biphasic paste - a mixture of corn syrup, water, and fine limestone, which they propose could be used as a reference paste. Ferraris *et al.* [131] have obtained rheological parameters for this paste using several different types of rheometers. Therefore, if the rheological properties of the reference paste are measured and compared to the published results prior to any rheometry investigation of biphasic pastes, it may indicate any deviations or errors and highlight issues with the rheometer, geometry, or protocol used that needs addressing.

In addition, the measurements of flow properties vary depending on the type of flow imposed on the paste (e.g. simple shear, uniaxial extensional, or oscillatory deformation) [113]. This highlights a further issue: rheometry of CaP pastes and cements are commonly conducted using rotational rheometers and it has not been established if the results obtained are representative of the flow characteristics observed during extrusion. However, methods to obtain rheological properties directly from extrusion tests are available.

10 Extrusion of Pastes: Benbow-Bridgwater Approach

Faced with the considerations outlined above, Benbow and Bridgwater [132] developed a semi-empirical approach to describe paste flow into and along a die that

enables useful rheological parameters to be obtained from extrusion testing of pastes. The parameters obtained could be applied to other extrusion configurations, for design, as well as allowing the effect of extrusion conditions and paste formulation to be compared.

The model assumes the pressure (P_{ex}) required to extrude the paste through the extruder comprises of two components: (i) the pressure difference (P_1) required to shape the paste from the barrel into the die land, and (ii) the pressure drop (P_2) to overcome wall friction as the paste travels along the die, Fig. 8.

For shaping the paste into the die land the paste is assumed to behave as a perfect plastic, where the work done in the die entry region is approximated by that required to achieve a homogeneous compressive deformation,

$$W = \sigma_y A_0 l_0 \ln \left(\frac{A_0}{A} \right) \quad \text{Eq. 13}$$

where σ_y is uniaxial yield stress, A_0 and A are the areas of the barrel and die, respectively, and l_0 is the length of a paste element within the barrel. The pressure difference to shape the material at the die land is the work done per unit volume, giving

$$P_1 = \sigma_y \ln \left(\frac{A_0}{A} \right) \text{ or } P_1 = 2\sigma_y \ln \left(\frac{D_0}{D} \right) \quad \text{Eq. 14}$$

In the die land is assumed to travel in plug flow (the bulk of the material flowing at a constant velocity) with only a very thin layer at the wall subject to shearing. The force required to enable the paste to flow through the die has to overcome the wall friction, which is the product of the wall shear stress and die perimeter area:

$$F_2 = \frac{P_2 \pi D^2}{4} = \tau_w \pi D L \quad \text{Eq. 15}$$

where τ_w is shear wall stress. Rearranging Eq. 15 gives

$$P_2 = 4\tau_w \left(\frac{L}{D} \right) \quad \text{Eq. 16}$$

The extrusion pressure can thus be written as

$$P_{ex} = 2\sigma_y \ln \left(\frac{D_0}{D} \right) + 4\tau_w \left(\frac{L}{D} \right) \quad \text{Eq. 17}$$

Velocity dependencies often arise in practice, which is incorporated in the expression for σ_y and τ_w , giving either the six-parameter model

$$P_{ex} = 2(\sigma_0 + \alpha V^m) \ln \left(\frac{D_0}{D} \right) + 4(\tau_0 + \beta V^n) \left(\frac{L}{D} \right) \quad \text{Eq. 18}$$

where V is the velocity of paste in the die land, or, when linear dependency is observed, the four-parameter model:

$$P_{ex} = 2(\sigma_0 + \alpha V) \ln \left(\frac{D_0}{D} \right) + 4(\tau_0 + \beta V) \left(\frac{L}{D} \right) \quad \text{Eq. 19}$$

The parameters σ_0 , α , m , τ_0 , β , and n are obtained by conducting extrusion tests at various ram velocities and using different extruder geometries. It can be seen that Eq. 18 and 19 can be used to estimate of the effect of syringe and needle geometry on injection performance. A second benefit of this approach is it allows the effect of different paste formulations and extrusion processes to be assessed and compared. Table 4 summarises paste systems and applications where this method has been used successfully [133–155] (Table 4).

Similarities exist between several of the extrusion studies presented in Table 4 and extrusion studies of CaP pastes and cements. Zhou and Li [137] used the Benbow-Bridgewater approach for the extrusion of short fibre reinforced construction cement.

The similarities between unset CPC and construction cements have previously been established [91]. This indicates the Benbow-Bridgewater approach can be also used for CPC. A major difference between the study by Zhou and Li [137] and CPC studies is the extruder geometry used by Zhou and Li [137] was considerably larger (barrel and die diameters of 80 and 12 mm) than syringes used in CaP cement and paste extrusion studies. In CaP cement and paste extrusion studies barrel diameters are commonly 11.5-15.4 mm (representative of 5 and 10mL syringes) [36,77,81,87,110], however barrel diameters from 4.5 [35] to 24 [78] mm have been used. Die (barrel exit) diameters reported range from 1.75 to 3 mm [35,36,87,110]. Die diameters can be further reduced if needles are attached. Ram (plunger) velocities used in CaP paste and cements extrusion studies generally fall in the range of 0.08 to 0.33 mm/s [36,77,87,110]. Considering syringe geometry this corresponds to flow rates of 0.012 to 0.048 mL/s. However due to variation in extruder geometry flow rates as low as 0.006 mL/s [35] and as high as 0.905 mL/s [78] have been used.

Martin *et al.* [134] used a barrel diameter of 25 mm when extruding a talc based hydraulic paste (SVF \approx 0.5, mean diameter of talc powder = 7 μ m). Various die diameters were investigated including diameters of 1, 2 and 3 mm. The ram velocities reported ranged from 0.05 mm/s to 2 mm/s (corresponding to flow rates of 0.0245 to 0.982 mL/s). Similarly to CaP pastes and cements, Martin *et al.* observed significant phase separation at the lower ram velocities investigated [134].

10.1 Limitations of the Benbow-Bridgewater Approach

The Benbow-Bridgewater model proved to be a successful and simple technique to obtain parameters concerning paste characteristics and extrusion conditions for design purposes [156]. However, several limitations exist:

- The actual die entry pressure drop is greater than that of the model prediction. As the deformation is not homogeneous, work is expended in shearing the material without contributing to the reduction in cross-sectional area [156,157] and shearing was not considered in this part of the model, Eq.17.
- Shearing is partially considered in Eq. 18 due to the dependence of velocity within the yield stress term, $\sigma_0 + \alpha V^m$. However, several authors [156,158,159] have highlighted that dealing with shear rate in this way neglects a term representing the geometry of the extruder, as the unit of measurement for shear rate is s^{-1} , whereas for velocity it is ms^{-1} . Therefore, if Benbow-Bridgewater parameters are obtained from an extruder of set dimensions, they may only be used to predict material behaviour on extruders of similar dimensions. To rectify this limitation, Zheng *et al.* [159] proposed that Eq. 18 should be modified as follows:

$$P = 2 \left(\sigma_0 + \alpha \frac{V^m}{D} \right) \ln \left(\frac{D_0}{D} \right) + 4(\tau_0 + \beta V^n) \left(\frac{L}{D} \right) \quad \text{Eq. 20}$$

Basterfield *et al.* [160] presented an alternative result for the P1 term in which the Gibson model [116] was rewritten in terms of the Herschel-Bulkley model, Eq. 12. This model assumes a radially converging flow pattern at the die entry, characterised by entry angle θ :

$$P = 2\sigma_0 \ln \frac{D_0}{D} + zk_u \left(\frac{2V}{D} \right)^n \left(1 - \left(\frac{D}{D_0} \right)^{3n} \right) \quad \text{Eq. 21}$$

where

$$z = \frac{2}{3n} (\sin \theta (1 + \cos \theta))^n \quad \text{Eq. 22}$$

Like the Benbow-Bridgwater approach, this has been used to obtain rheological parameters directly from extrusion data of several paste formulations [161–163]. It also has limitations, including the absence of the work associated with the transition into and out of the radial flow, the absence of wall shear, and the deviation of the assumed flow pattern from those observed in flow visualisation studies [164,165].

These shortcomings in both the Benbow-Bridgwater and the Basterfield *et al.* models highlight the complexity of the flows, which are the subject of detailed computational investigations [164,165]. They do, nevertheless, provide a systematic framework for understanding the factors affecting extrusion and quantifying the impact of these.

Ferstl *et al.* [148] and Liu *et al.* [140] observed a limiting velocity when obtaining Benbow-Bridgwater parameters for the paste formulations they investigated: below this velocity phase separation occurred. As with CPC, the phase separation caused the paste in the extruder to dewater, increasing the required extrusion pressure. If phase separation occurs, the Benbow-Bridgwater or Basterfield approach cannot be used to obtain rheological parameters.

11 Phase Separation during Extrusion of Pastes

Phase separation is not limited to CaP based cements and pastes. It has also been encountered during extrusion of various biphasic pastes in the chemical, food and pharmaceutical sectors. Phase separation is not only detrimental to the quality of extrudate; it can also be very damaging to the extrusion system, depending on materials used and extrusion pressure. These factors have stimulated numerous investigations into how to reduce phase separation for all the above sectors. The approaches taken to investigate phase separation in these studies were similar to those of Habib *et al.* [34]. The quality of extrudate and paste in the barrel were

analysed, post and also during extrusion [134,140,166–169]. Additionally, some studies have been able to observe both liquid distribution of paste within the barrel during extrusion and flow of the powder component [107,164,165]. Recently, O'Neill *et al.* attempted to achieve this for the extrusion of CaP pastes [36]. The flow of the powder component of the CaP paste (β -TCP) was determined using powder tracing tests and the flow of the liquid was inferred by determining the lateral and radial liquid distribution within the syringe barrel. Important flow characteristics such as the existence of static zones at the lateral wall of the barrel, that readily released liquid exacerbating phase separation, were identified [36].

Like the study by Bohner and Baroud [78], several studies have taken a theoretical approach in an attempt to gain a deeper understanding of the phase separation process and identify the mechanism and extent by which different methods may reduce phase separation [150,151,170,171]. As a result, the understanding of phase separation during extrusion of these pastes is more advanced compared to the extrusion of CPC.

Several phase separation mechanisms have been identified in different research studies: (i) *filtration* in the barrel, where the pressure exerted by the plunger on the paste causes drainage of the liquid phase and the solids to undergo consolidation [152,172]; (ii) *suction*, driven by dilation of the powder network as it flows into the die [170]; and (iii) *filtration in the needle* (or die land), exacerbated with the formation of solid mats [171] (Fig. 9).

Similar to the extrusion of CPC, there are a number of factors affecting phase separation of other biphasic pastes. To eliminate phase separation, these factors have to be optimised within the constraints of the considered application. Although other

extrusion applications may not be as highly constrained as the extrusion of CPC for minimally invasive surgical applications, optimising paste formulation and extrusion parameters by experimental methods alone is still difficult. For this reason theoretical models describing the three mechanisms have been developed.

11.1 Phase Separation by Filtration: Mechanism (i)

During extrusion, pressure is applied to the paste through the plunger to shape the paste into, and push it through the die. If the SVF is high enough to form a connected network, the total extrusion pressure is the sum of pressure imposed on the particle network and interstitial liquid [151]. If the pressure imposed on the liquid (called pore pressure in soil mechanics), is high relative to the permeability of the particle network, phase separation will occur [151]. The liquid will migrate through the network to regions of lower pressure, i.e. out of the extruder, resulting in a faster flowing liquid relative to the powder and a liquid-rich extrudate.

Khelifi *et al.* [150] and Rough *et al.* [151] used similar approaches to model this phase separation mechanism. For modelling purposes the paste within the barrel was considered as two separate regions: (1) the paste at the barrel exit or shaping zone, and (2) the paste upstream of the shaping zone in the remaining volume of the barrel. The paste in the shaping zone region was assumed to be homogeneous. Using the Benbow-Bridgewater approach the pressure required to extrude this paste was calculated. The paste upstream of the shaping zone was assumed to undergo plug flow and transmit the pressure exerted by the plunger to the paste at the shaping zone. It was in the region upstream of the shaping zone where liquid migration was modelled. Using a soil mechanics approach the pressure imposed on the solids and the pore pressure was calculated. A Darcy-like liquid flow through the permeable powder

network was modelled. The paste was considered in separate layers (50 [150] and 12 [151] layers). It was assumed that liquid filtered from each layer travelled to the layer below. Following every modelling iteration, each layer had a new SVF and the bottom layer was assumed to be extruded; the SVF of the bottom layer was then used to predict the extrusion pressure. This process was repeated until the final layer was extruded. The pressure gradient in the barrel due to friction between the paste and extruder wall also considered.

Both studies employed empirically fitted parameters and values taken from soil mechanics literature. There was good agreement between the model predictions and the experimental observations, considering the level of approximation [150,151]. Rough *et al.* claimed the main inconsistency in the model was its inability to accurately predict the water content of the paste (a microcrystalline cellulose paste) at the die face. The measured paste liquid content was lower than the model predictions, attributed to the presence of static zones and suction effects resulting from dilation of the solids [151], i.e. mechanism (ii), which their 1-D model did not consider.

11.2 Phase Separation by Dilation: Mechanism (ii)

In concentrated pastes, powders are close to the SVF_{max} . To enable flow, particles must move apart to move past each other. This requirement is particularly evident during extrusion where particles in a highly concentrated paste within the extruder barrel are closely packed and have to move apart to flow through the die entry, i.e. the powder network must dilate. The dilation of the particle network results in an increase in voidage, reducing pore pressure (suction). This negative pore pressure, relative to the pore pressure of neighbouring regions, draws liquid from those regions through the particle network, resulting in a liquid rich extrudate. Patel [172] simulated extrusion

of a paste, including pore suction, in a 2D finite element model based on soil mechanics theory. Stresses applied to the paste were divided between the liquid and powder network. The powder network was modelled using a modified Cam-Clay constitutive model, commonly used in soil mechanics. Liquid migration was predicted using Darcy's law. The stress applied to the liquid and powder network, and the distributions of the two phases, was calculated throughout the barrel and along the die land. This allowed the effect of dilation at the barrel exit on the pore pressure of the paste in the surrounding area to be assessed.

There was reasonable agreement between the model predictions and the experimental observations at low SVF, however agreement was poor at high SVF [172]. This study relied heavily on parameters taken from soil mechanics literature. The model paste studied consisted of 140 μm diameter glass spheres in a viscous aqueous glucose solution. Specialised characterisation techniques were required and some effort would be required to develop these for CPC and CaP materials. Their work nevertheless demonstrates that extrusion of biphasic systems subject to phase separation in geometries of interest to the field can be modelled using existing numerical techniques.

11.3 Filtration in the Needle: Mechanism (iii)

Yaras *et al.* [171] observed that phase separation of a concentrated paste (aluminium, ammonium sulfate and hydroxyl terminated polybutadiene) was exacerbated by the continual formation and break-up of mats of solids at the die. The extrudate became liquid rich and dry in a cyclic pattern and was extruded out in bursts [171]. This resulted in a peak- and trough-type plunger force profile due to formation and break-up of mats of solids, as well as an increase in required extrusion force due to dewatering of the paste within the barrel.

Yaras *et al.* [171] did not model the formation and break-up of mats of solids directly. The simple model produced was based on competition between the velocity of the bulk paste flowing through the die and velocity of liquid draining through the powder network. A Darcy-type flow through the powder network was assumed. Several assumptions were made, including the pressure used to predict the flow of liquid through the powder network was assumed to equal the pressure to overcome the paste-wall friction within the die¹, similar to the model proposed by Bohner and Baroud [78]. The phase separation predictions were reasonable considering the simplicity of the Yaras *et al.* model [171].

12 Phase Separation Mechanism of Calcium Phosphate Pastes and Cements

Considering the different possibilities reviewed above, it is evident that knowledge of the phase separation mechanism is required when applying theoretical approaches to describe experimental observations. A better theoretical understanding is essential to enable efficient optimisation of CaP pastes and CPC systems and to fully satisfy clinical requirements for surgical applications requiring extrusion or injection.

When investigations into the injectability and phase separation of CaP pastes are cross-compared, common features exist that indicate the occurrence of a similar phase separation mechanism, or mechanisms. The common features that have been observed are: (1) similar extrusion pressure profiles, initially exhibiting a slight gradient or plateau followed by an abrupt increase in extrusion pressure [34–36,79,87]; (2) an

¹ It can be shown that the two pressures cannot be equal, as the work associated with the flow of liquid through the powder is dissipated against the fluid viscosity, so that no energy is then available to drive the solids along the wall. Likewise, if there is no fluid drainage, the pressure is equal to the work required to extrude the solids and any entrained liquid. It does, however, provide a reasonable estimate of the maximum drainage rate.

extrudate with a higher LPR than the initial paste which remains relatively constant throughout extrusion [34–36] and (3) paste remaining in the barrel having a lower LPR than the initial paste, which is drier on the plunger side of the barrel compared to the exit side [34,36]. In addition, needle geometry does not appear to have a strong influence on phase separation during extrusion of CaP pastes [34,36,87]. However, alteration of the barrel geometry has been observed to significantly affect phase separation [36]. This indicates that the phase separation is located within the barrel, i.e. mechanism (i) or (ii). Differentiating between these two mechanisms is difficult. In a recent study, O'Neill *et al.* [36] proposed that phase separation by dilation may be the dominant mechanism. They observed that the extrudate LPR remained relatively constant throughout the extrusion process, indicating that a critical LPR had to be reached to enable the paste to exit the barrel. The extrudate LPR did not appear to be dependent on extrusion pressure, suggesting mechanism (i) was not the dominant mechanism. Therefore, it would be reasonable to assume the required dilation of the paste at the barrel exit to reach the critical LPR would create a suction pressure, drawing the liquid through the powder matrix, i.e. mechanism (ii). This would result in the paste remaining in the barrel being steadily dewatered and increasing extrusion pressure as the minimum LPR of paste remaining in the barrel is reached.

Interestingly, although Montufar *et al.* observed the needle geometry had no effect on the extrusion of CaP pastes, an effect was observed during the extrusion of CPC [87]. Reducing the inner diameter of the needle significantly reduced the injectability of CPC. Episodes of clogging of the needle were evident on the extrusion profile for CPC [87]. This may be comparable to the formation of mats observed by Yaras *et al.* [171] and therefore suggests that when a setting reaction is present mechanism (iii) may occur, in addition to the phase separation occurring in the barrel, i.e. mechanism (i) or

(ii). Mats are known to form when non-spherical particles are extruded, via solid bridging, and becomes increasingly important when the particle sizes approach the dimension of the needle [173].

Further work is required to confirm the phase separation mechanisms present during the extrusion of CaP pastes and cements, and the extent various factors influence these mechanisms.

13 Concluding Remarks

CPC have seen clinical success in many dental and orthopaedic applications due to their ability to be moulded into bone defects and implant sites, then harden *in situ* to provide stability. However, there is limited clinical use of CPC in surgical applications requiring extrusion or injection due to their relatively poor injectability.

Poor injectability of CPC primarily arises from the separation of the solid and liquid phases during cement delivery from a syringe/cannulated needle arrangement, which was the focus of this review. Several methods to reduce the phase separation of CaP materials during extrusion have been established, which include: increasing LPR and injection rate, reducing plastic limit, increasing the viscosity of the liquid component or the repulsive force between particles, optimising barrel geometry, and agitating paste throughout extrusion. However, use of these methods is limited by the highly constrained nature of surgical applications and the fact that these improvement methods are usually detrimental to other crucial CPC properties. These constraints, and the number of factors potentially influencing phase separation, have hindered optimisation of an injectable CPC that fully meets the necessary requirements for minimally invasive surgical applications.

Future formulations of CPC are likely to rely on the application of additives in an attempt to meet all clinical requirements, i.e. to reinforce CPC and improve mechanical performance, ensure cohesion during setting and enhance interconnected macroporosity. It has been observed that additives, whether powder or liquid, can have a detrimental or positive influence on the delivery process of CPC. Further investigations into how the size and proportion of solid additives relative to the bulk CaP powder can be used to optimise packing ability of the powder should be conducted. This will increase SVF_{\max} (i.e. reduce plastic limit) and reduce phase separation during injection/extrusion. When using liquid or readily soluble additives, the influence on viscosity of the liquid component should be considered. A viscous liquid has been shown to reduce phase separation during injection/extrusion of CaP pastes and cements, but increasing viscosity of the liquid too much will increase extrusion force, which may exceed the force the surgeon is able to apply and still maintain sufficient control. Therefore, investigations into the optimal viscosity of the liquid component should be conducted in the future.

In addition, as optimisation of CPC by experimental work alone has proven extremely difficult, greater focus should be on increasing the theoretical understanding of the phase separation of CPC during extrusion/injection. Development of accurate theoretical and computational models would improve the efficiency of the optimisation process. Indeed, several models describing phase separation mechanisms during the extrusion of biphasic pastes do exist. However, due to lack of knowledge surrounding the phase separation mechanisms occurring during the extrusion of CPC, and the fact that rheology and permeability of CaP paste systems are relatively under characterised, application of these models for CPC has been limited. Although the understanding of phase separation has greatly improved recently, research is still

needed to confirm the exact phase separation mechanisms that are present during the extrusion of CaP materials and the extent various factors influence these mechanisms; only then can accurate models become a realistic prospect.

In addition, obtaining reliable parameters representing material characteristics such as permeability and rheological properties of the paste system is required when attempting to model the extrusion or phase separation process. Several studies have made progress in this area by investigating factors influencing the rheology of CaP cements and pastes. However, due to a lack of established protocols, comparison of rheological properties of different paste formulations between separate studies has been difficult. When obtaining parameters intended for inter laboratory use, it is imperative to know if variation observed between measurements is due to differences in material characteristics or differences in experimental protocol. Therefore, standardised protocols need to be established. It is evident from the more established field of cement rheology that it is extremely difficult to obtain absolute or even reliable and repeatable data, largely due to the complexity and inherent heterogeneity of cement based materials. Consequently, a considerable effort is required to standardise the methodologies and protocols used to determine the rheological properties for all cement like pastes, not just CPC based systems.

Furthermore, with respect to phase separation occurring during extrusion of CaP materials, little work has been conducted to determine the extent permeability and rheological properties of CaP pastes and cements influence phase separation. In addition limited research has been conducted to directly measure factors influencing permeability of CaP paste systems. This should also be addressed in future studies.

In summary, obtaining parameters that represent the permeability and rheological properties of CaP pastes and establishing the influence these properties have on the extrusion process is needed to improve the understanding of the phase separation mechanisms occurring during extrusion of CaP pastes and cements. It would also enable specific parameters to be tested with computational models describing the extrusion process of biphasic pastes to establish if these models can be used or modified to accurately describe the extrusion process of CaP pastes and based cements. A greater understanding of, and ultimately a rigorous theoretical model describing, the extrusion process of CaP pastes and cements will greatly aid studies attempting to optimise fully injectable CaP pastes or cements that fully meet clinical requirements for minimally invasive surgical applications.

14 References

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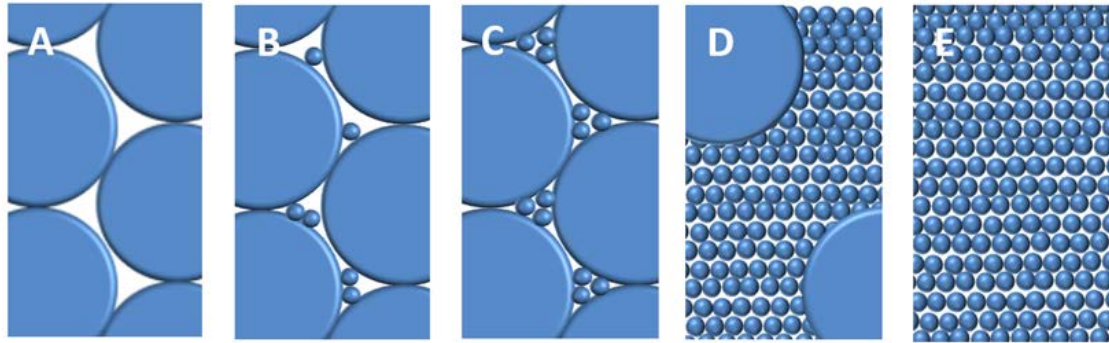


Fig. 1: Packing fraction of mono- (A & E) and bi-dispersed (B, C & D) particles.

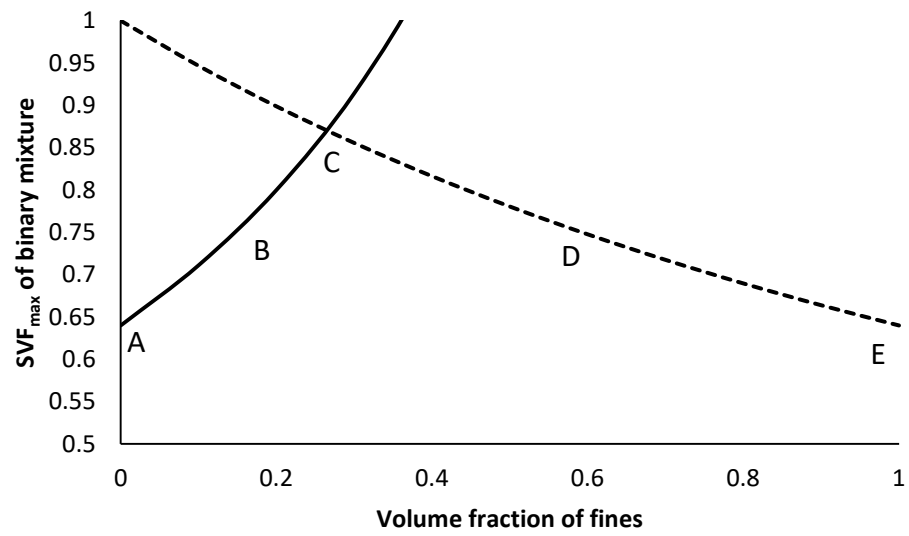


Fig. 2: Calculated results from the Furnas equation. The solid line represents Eq. 4i, dashed line represents Eq. 4ii. $SVF_{\max f}$ and $SVF_{\max c}$ values used were 0.64. The letters relate to schematics presented in Fig. 1.

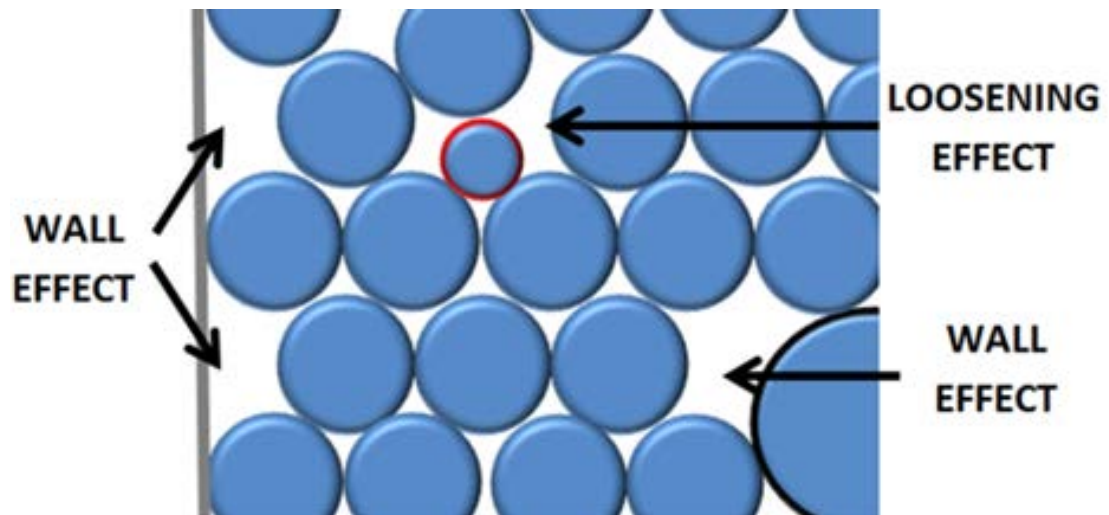


Fig. 3: Packing structure disrupted by the loosening and wall effect.

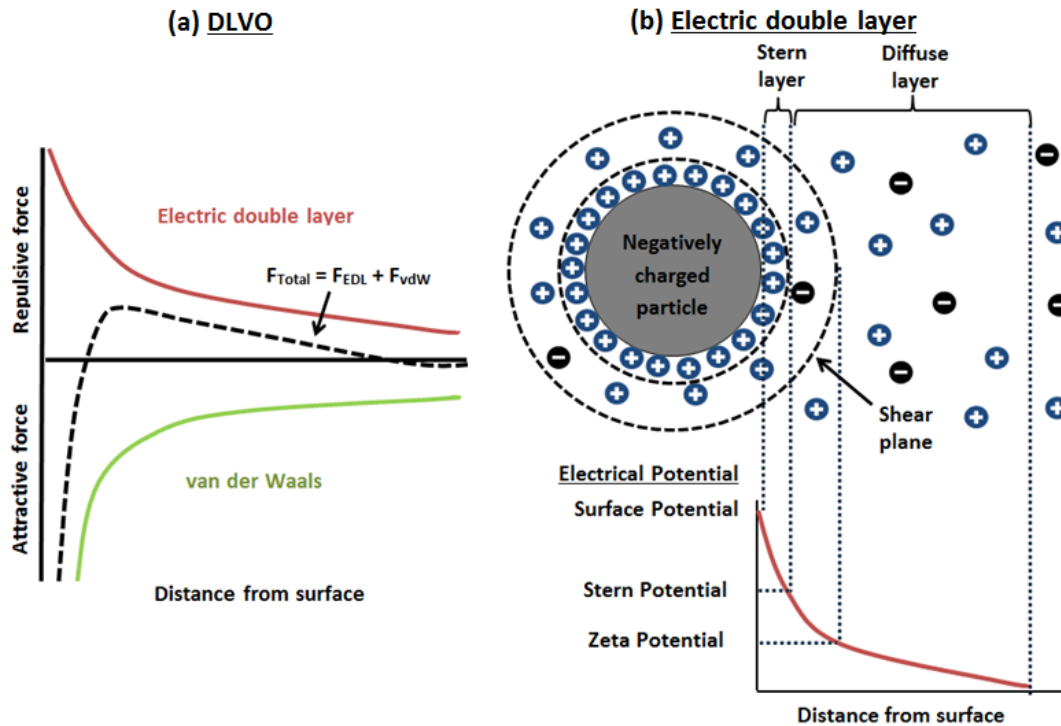


Fig. 4: (A) Representation of DLVO theory- forces are simplified to the sum of the attractive Van der Waals and repulsive EDL forces, (B) schematic of the EDL, consisting of an inner (Stern) layer where counter-ions are strongly bound, and an outer (diffuse) layer where concentration of counter-ions decreases with distance, until equilibrium is reached. Within the diffuse layer there is a shear plane inside which counter-ions move with the particle. The potential at this boundary is the ζ P.

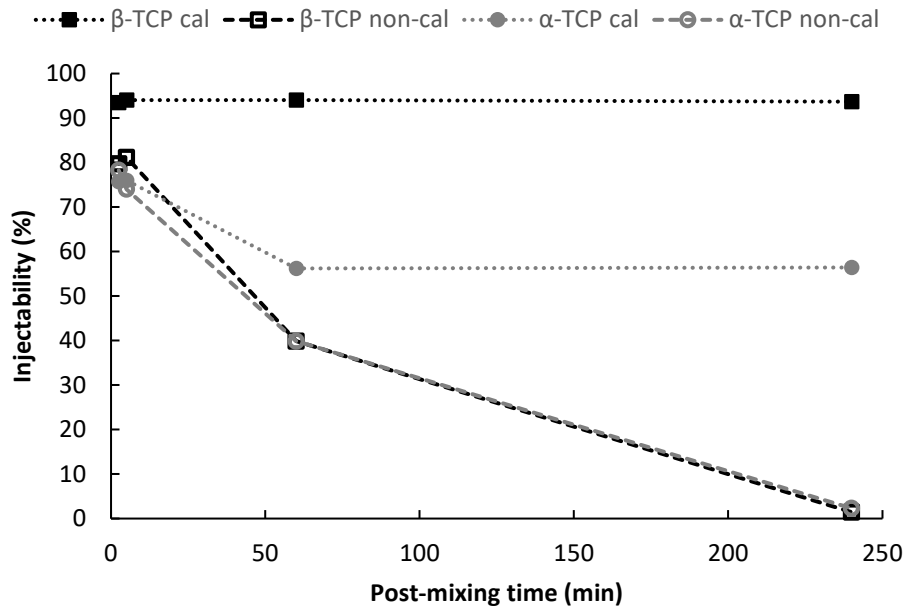


Fig. 5: Injectability and of α - (circles) and β - (squares) TCP (LPR of 0.45) either non-calcined (open symbols) or calcined (close symbols), as functions of post-mixing time. Initial setting times of non-calcined α - and β -TCP were 35 ± 2 and 52 ± 3^2 min respectively. Reproduced from [57].

² The apparent setting reaction of the β -TCP, previously assumed to be non-setting, was thought to be due to partial amorphisation during milling. After calcination at 500 °C, no amorphous phase was detected in the β -TCP powder and injectability of pastes produced from calcined β -TCP, remained constant throughout the 240 min.

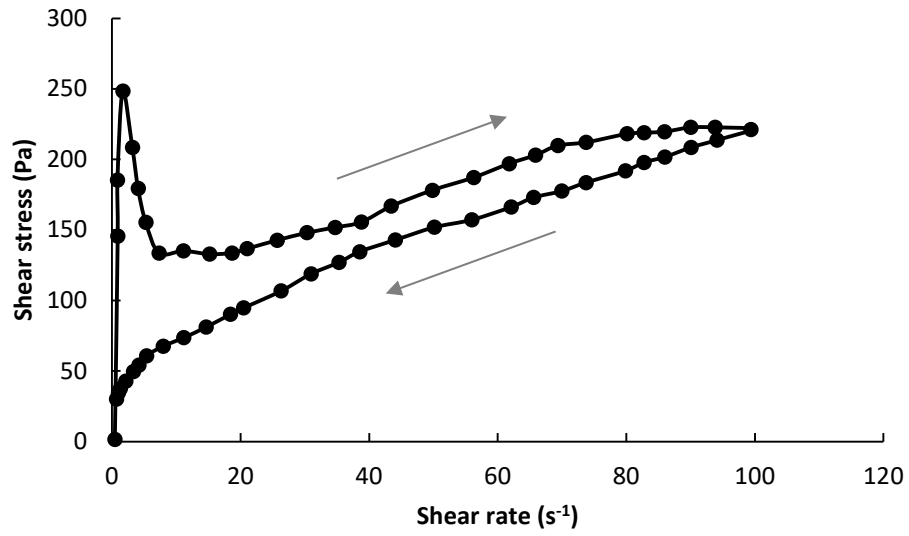


Fig. 6: The relationship between shear stress and shear rate of calcium phosphate cement, 0.5 min after mixture of the powder with deionised water. Arrows indicate outward and return sweeps. Hysteresis loop indicative of thixotropy. Reproduced from [88].

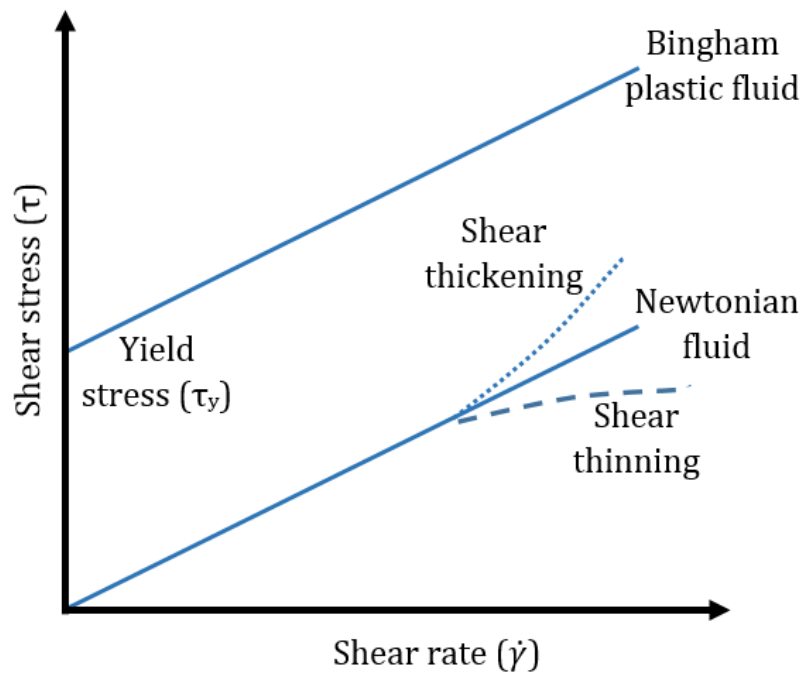


Fig. 7: Classification of fluids, based on relationship between shear stress and shear rate.

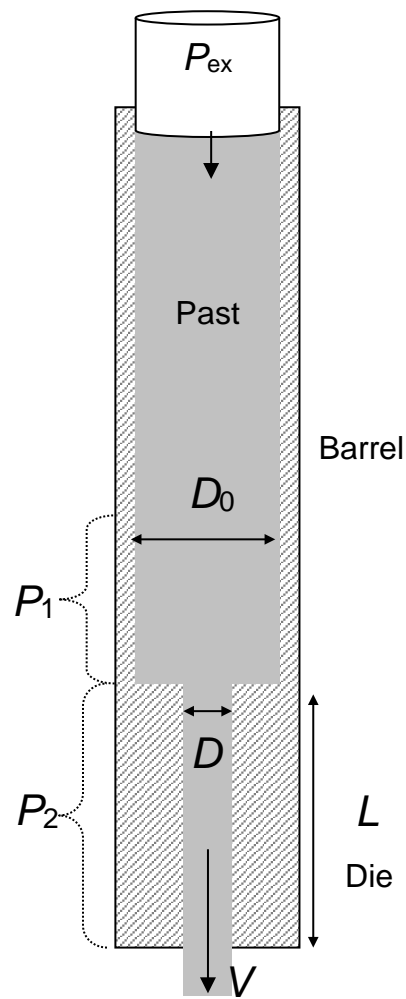
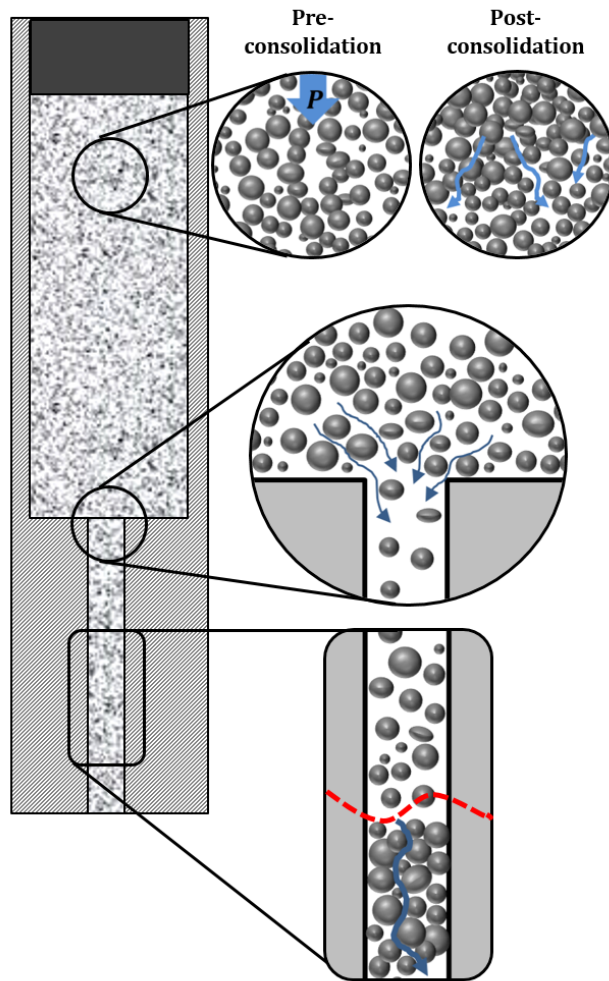


Fig. 8: Schematic of ram extruder configuration. Barrel diameter D_0 , die diameter D and length L , at a mean extrudate velocity, V . Adapted from Wilson and Rough [98].



Mechanism (i): Filtration.

When the pressure, P , exerted on a paste is high relative to the permeability of the paste system, consolidation of the powder phase and drainage of the liquid phase occurs.

Mechanism (ii): Suction.

If the solid volume fraction of a paste is sufficiently high, particles must move apart to enable flow, i.e. the powder must dilate. Liquid is then drawn through the powder network to fill voids created by

Mechanism (iii): Filtration in the needle.

Above the red dotted line represents normal flow in die, below the same line shows the formation of a solid mat or powder blockage. As the powder is static or slow moving in the die, but the plunger is still moving, the liquid must flow through the

Fig. 9: Phase separation mechanisms observed during extrusion of pastes and their location in the extruder. Schematic diagram is not to scale.

able 1: A selection of animal studies investigating the resorption rate of different calcium phosphate cement based systems.

Author	Method	Maximum Duration	Calcium Phosphate Cement Type	Volume Resorbed (%)
Apelt <i>et al.</i> [17]	Cement sample implanted in cylindrical bone defects (Ø 8mm, 13mm deep) of sheep.	6 month	ChronOS Inject (brushite)	60
			Experimental cement (brushite)	90
			Norian SRS (apatitic)	Bulk remained
Ooms <i>et al.</i> [18]	CPC injected as a paste into cortical bone defects of goats (Ø 5mm).	24 weeks	Norian SRS (apatitic)	Bulk remained
Knaack <i>et al.</i> [19]	Femoral slot (0.5 by 4 to 6 mm) defects in dogs were filled with autologous bone implants or CPC.	26 weeks	Alpha-BSM (apatitic)	>99
Miño-Fariña <i>et al.</i> [20]	CPC injected into bone defect (Ø 6mm, 8mm deep) of rabbits.	12 weeks	Macroporous cement (apatitic)	65

Table 2: Comparison of the maximum compressive load (MCL) between initial and treated vertebrae (VB) of the thoracic and thoracolumbar regions in four cement groups (Data are presented as means \pm SD). Adapted from [28].

	Cement Group	Compressive Strength (MPa)	# VBs	Initial MCL (N)	Treated MCL (N)	<i>p</i> value
Thoracic spine	CaP-1	5	10	2022 \pm 800	1676 \pm 596	0.009
	CaP-2	20	9	2438 \pm 928	1906 \pm 452	0.017
	CaP-3	50	10	2023 \pm 806	1829 \pm 821	0.177
	PMMA	80	9	1865 \pm 649	2899 \pm 795	0.000
Thoracolumbar spine	CaP-1	5	9	2878 \pm 855	2546 \pm 912	0.045
	CaP-2	20	9	3367 \pm 1098	2918 \pm 798	0.053
	CaP-3	50	8	3168 \pm 1302	2710 \pm 947	0.055
	PMMA	80	9	3239 \pm 1177	4752 \pm 1436	0.004

Table 3: Selection of studies investigating the addition of secondary powders or beads to CaP (primary) powder.

Author	Primary powder	D ₅₀ of primary powder (µm)	Secondary powder or beads	D ₅₀ of secondary powder (µm)	Quantity of secondary powder added (wt%)	Findings
Gbureck <i>et al.</i> [61]	α-TCP	9.84	DCPA	1.161	23	Significantly improved paste injectability and reduced water demand. Max. powder-liquid ratio was 3.5 g/mL for α-TCP and 5, 4.5 and 4 g/mL for DCPA, TiO ₂ and CaCO ₃ mixtures, respectively.
			TiO ₂ ,	0.554	23	
			CaCO ₃	0.724	23	
Tadier <i>et al.</i> [14]	β-TCP	1.8	Glass beads	156 and 390	13, 25, 36.5, 42 and 45.6	Addition of 156 µm beads improved injectability. Addition of 390 µm beads had little effect on injectability. However, 42 wt% and 45.6 wt%, was did not inject. Attributed to beads forming a percolating network.
Qi and Ye [62]	Part crystallised CaP and DCPA	-	PLGA microspheres	100-200	10, 20, 30 and 40	The addition of the microspheres decreased injectability.

Table 4: Examples of paste materials characterised using the Benbow-Bridgewater model from 2000 onwards. Adapted and updated from Wilson and Rough [98].

Material	Researcher (Year)
Agro-chemical pastes	Martin <i>et al.</i> (2004) [99]
Catalyst monoliths	Das <i>et al.</i> (2002) [100] Yekta (2007) [101]
Cement based material	Zhou and Li (2005) [102]
Ceramics	Bates and Bridgewater (2000) [103] Guilherme <i>et al.</i> (2009) [104] Liu <i>et al.</i> (2012) [105] Raupp-Pereira <i>et al.</i> (2007) [106] Ribeiro <i>et al.</i> (2004) [107] Vitorino <i>et al.</i> (2014) [108] Wells <i>et al.</i> (2005) [109]
Detergent	Russell <i>et al.</i> (2004) [110]
Fuel cell monoliths	Powell and Blackburn (2010)[111] Powell <i>et al.</i> (2013) [112]
Hard metal paste	Ferstl <i>et al.</i> (2012) [113]
Pharmaceutical pastes	Bryan <i>et al.</i> (2015) [114] Khelifi <i>et al.</i> (2013) [115] Rough <i>et al.</i> (2002) [116] Zhang <i>et al.</i> (2011) [117]
Rocket propellants	Wight and Reed (2002) [118]
Snack food dough	Cheyne <i>et al.</i> (2005) [119]
Zeolite adsorbents	Li <i>et al.</i> (2001) [120]